GUIDANCE DOCUMENT

FOR SAMPLE COLLECTION AND THE USE OF COMMERCIAL PRESENCE-ABSENCE (P-A) TESTS FOR THE BACTERIOLOGICAL ANALYSIS OF DRINKING WATER

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PREAMBLE

Suppliers of food and drink for human consumption have a special responsibility under the law to do everything reasonably possible to ensure that their products are fit to be consumed. Drinking water treatment plant owners and drinking water distribution system owners are responsible for ensuring that the water delivered to the consumer is potable and that the water is tested regularly and properly for concentrations of chemicals which may be harmful and for bacteria which indicate whether or not the water is safe for human consumption. This document provides information about presence-absence (P-A) test methods for detecting bacteria indicative of faecal contamination in drinking water, procedures for sample collection and protocols for the use of commercially available P-A methods considered acceptable for testing drinking water. In addition, it provides guidance for the collection and handling of samples for heterotrophic plate count (HPC) analysis and guidance for handling samples collected from the raw (source) water.

DISCLAIMER

It is not within the mandate of the Ontario Ministry of Environment and Energy or the Ontario Ministry of Health to endorse any particular commercial product or service mentioned in this document.

References to any commercial products or services are strictly for the purpose of discussion and guidance for their use.

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1.0 BACKGROUND INFORMATION

Section 52:1 of the Ontario Water Resources Act, R.S.O. 1990, stipulates that the Ministry of Environment and Energy (MOEE) has a mandate to issue approvals for the establishment, alteration, extension or replacement of water works that are capable of supplying more than 50,000 litres of water per day or that serve more than five residences. Many Certificates of Approval (C's of A) issued by the MOEE outline the frequency and types of tests which, at minimum, must be performed on the raw (source) water, in process water and/or the finished drinking water. Many C's of A also stipulate that the methods used to test drinking water must be consistent with those contained in the most recent American Public Health Association, American Water Works Association and Water Environment Federation publication titled "Standard Methods for the Examination of Water and Wastewater" or must be acceptable to the MOEE. Furthermore, a record of all analytical results must be maintained at the treatment plant and must be available for inspection by MOEE abatement officers.

In the past, many municipalities used provincial government laboratories to analyze samples and ensure that the water met the Ontario Drinking Water Objectives (ODWO). However, the Ministry of Environment and Energy (MOEE) and the Ministry of Health (MOH) in 1996 advised water works owners that provincial government laboratories would no longer test the drinking water samples routinely collected from community supplies. Water works owners, who had sent samples to provincial laboratories, were notified to make alternate arrangements for routine drinking water analyses and it was strongly recommended that they use accredited private laboratories certified to perform the required tests.

In response to these changes, water treatment plant operators and municipal, regional and private laboratories have asked whether or not commercially available Presence-Absence (P-A) tests are acceptable for testing drinking water for the presence of bacteria indicative of faecal contamination. In addition, water works owners have asked whether or not commercial P-A tests can be used at treatment plants to test drinking water samples since in-house testing provides some advantages. The time between sampling and analysis will be reduced and water works staff will observe the results, notify appropriate authorities and respond to adverse conditions more quickly than when having to wait for a report from an outside laboratory.

The coliform and Escherichia coli (E.coli) detection principles upon which commercially available P-A methods are based are included in "Standard Methods for the Examination of Water and Wastewater" and thus satisfy C of A requirements. In addition, bacteriological tests on drinking water should begin as soon as possible after sample collection. Furthermore, no regulation currently exists which prohibits water works owners from performing tests on drinking water at their own treatment plants.

Therefore, both the MOEE and the MOH agree that commercially available P-A tests may be used at municipal laboratories, private laboratories or water works facilities **to screen** samples routinely collected from community water supplies. However, the MOEE and MOH strongly

recommend that positive results be confirmed by submitting additional "special" samples (ODWO 1994, section 4.1.3.1) for thorough analysis to an accredited laboratory certified to perform bacteriological tests on drinking water.

In conclusion, the MOEE and the MOH recommend the following practises.

1) Laboratories or treatment plant testing facilities which perform tests on drinking water are strongly encouraged to become accredited by an agency such as the Canadian Association of Environmental and Analytical Laboratories (CAEAL) and certified for all tests they wish to perform in fulfillment of the requirements of the Ontario Drinking Water Objectives (ODWO).

2) Water works owners may use any combination of private or municipal laboratories or in-house testing facilities to satisfy analytical requirements as long as they use methods which are outlined in "Standard Methods for the Examination of Water and Waste Water" or methods which are acceptable to the MOEE.

3) Commercially available P-A tests may be used at private or municipal laboratories or at treatment plant testing facilities to screen drinking water samples for bacteria indicative of adverse drinking water quality.

4) Any positive result which occurs using a commercial P-A test must be confirmed by submitting additional "special" samples for thorough analysis to an accredited laboratory certified to perform bacteriological tests on drinking water.

5) Laboratories or treatment plant testing facilities should use properly trained staff and standard operating practices which encourage confidence in the accuracy and precision of their analyses.

6) Laboratories or treatment plant testing facilities must document any Quality Assurance/Quality Control (QA/QC) program in place at the laboratory or testing facility.

7) When samples are tested at a treatment plant facility, a record of QC results must be maintained along with the results of tests performed on water samples routinely collected from the distribution system.

8) Laboratories should, with permission of the treatment plant or distribution system owner, report results indicating the persistence of coliforms or the presence of E.coli to both the MOEE district abatement officer and the local Medical Officer of Health, the ODWO not withstanding.

The remainder of this document provides information about P-A methods, procedures for sample collection and protocols for the use of commercial P-A products available for testing drinking water for bacteria indicative of adverse conditions. In addition, it provides guidance for the collection and handling of samples for heterotrophic plate counts (HPC) and the handling of samples collected from the raw (source) water.

2.0 PRESENCE-ABSENCE (P-A) TESTING

The presence of any E.coli per 100 mL in any sample of drinking water from the distribution system or the presence of any coliform per 100 mL in consecutive samples from a single site, at multiple sites or in more than 10% of samples per month is unacceptable (ODWO 1994, section 4.1.2). Because it is necessary to know only whether or not E.coli or other coliforms are present per 100 mL, presence-absence (P-A) methods which test 100 mL volumes of drinking water can be used to determine compliance with the Ontario Drinking Water Objectives.

The MOEE and the MOH advocate the use of presence-absence tests for screening samples routinely collected from drinking water supplies because the tests are less costly and more sensitive than membrane filtration (MF) methods (1,2) for detecting bacteria indicative of adverse conditions in drinking water.

Quantitative methods (e.g. membrane filtration) are useful for special studies, testing water from new or repaired mains or complete sanitary surveys if the results of P-A tests indicate a continuing water supply problem.

2.1 Description of the MOEE P-A Test

The MOEE laboratory has used a Presence-Absence (P-A) test for over 25 years to screen drinking water samples quickly and inexpensively for the presence of bacteria indicative of unsafe or deteriorating water quality as outlined in the Ontario Drinking Water Objectives (3). The P-A broth (Difco) used by the MOEE is prepared at the laboratory. Therefore, the MOEE method is unsuitable for use by treatment plants that do not have the equipment for preparing and sterilizing culture media. The following information and description of the test is presented strictly as an introduction to the concept of P-A testing and the approach used by the MOEE laboratory.

The MOEE P-A method allows detection of total coliforms, faecal coliforms and E.coli which are used as indicators of faecal contamination. The method also allows detection of other bacteria which are considered undesirable in treated drinking water including Aeromonas species (spp.), Staphylococcus aureus, faecal streptococci, Pseudomonas aeruginosa and Clostridium spp. Many of these bacteria are opportunistic pathogens and their presence in drinking water is considered indicative of a deteriorating condition within the distribution network (ODWO 1994, section 4.1.4).

Because the MOEE P-A test allows detection of undesirable bacteria, in addition to E.coli and other coliforms, the method not only provides a way of detecting bacteria indicative of faecal contamination but also provides a way of detecting other bacteria which may accumulate as a result of inefficient treatment, inadequate distribution

system maintenance, biofouling or low disinfectant residuals. Prompt remedial action at the first sign of a deteriorating condition protects public health; may reduce the frequency of taste, odour and corrosion problems; may reduce the need for costly maintenance or repairs; may extend the life of the distribution system and helps operators to manage the system in an effective manner.

During testing, 100 mL of water sample is added to a bottle containing a liquid culture medium (P-A broth) which is then incubated for up to 3 days at 35°C ± 0.5°C. The detection of coliforms, E.coli and other undesirable bacteria is based upon the ability of many of these bacteria to ferment lactose. A change in the colour of the broth from purple to yellow at any time within 3 days incubation indicates adverse drinking water quality. Coliforms are very likely present if tapping or gentle shaking of a positive (yellow) P-A bottle causes tiny gas bubbles to effervesce from the broth.

Whenever a positive (yellow) reaction occurs, liquid from the positive P-A broth is transferred to other culture media (confirmation tests). The results of these tests are used to confirm the presence of coliforms, faecal coliforms, E.coli, Aeromonas spp, faecal streptococci or Staphylococcus aureus. The series of confirmation tests may also detect Pseudomonas aeruginosa and Clostridium spp.

Any positive (yellow) P-A reaction indicates, at minimum, a deterioration of drinking water quality. Treatment plant staff are notified as soon as bacteria from a drinking water sample cause the MOEE P-A broth to turn yellow. This is done so that staff can begin an investigation of the site, check disinfectant residuals in the area of the affected site, increase the disinfectant level if necessary, and collect additional "special" samples as soon as possible (ODWO 1994, section 4.1.3.1). Treatment plant staff must take corrective action immediately (ODWO, section 4.1.3) if confirmation tests indicate the presence of coliforms at multiple sites or any presence of E.coli. However, staff may take corrective action before receiving the results of confirmation tests particularly when positive (yellow) reactions occur in samples from multiple sites.

Corrective action must be taken immediately if tests on additional "special" samples reveal the presence of E.coli, coliforms or any other bacterial indicator of deteriorating drinking water quality. Basically, this means that corrective action must be taken if the P-A test on any "special" sample turns positive (yellow). By using this approach, adverse conditions can be detected effectively and action can be taken quickly without overreacting to the positive tests which may occur from time to time at individual sites within the distribution system.

2.2 Commercial P-A Tests

A number of presence-absence test products are now commercially available in kit form for testing drinking water. These include, but may not be limited to, the Hach Presence-Absence test, Colilert, Colisure and ColiBag. All of these products will detect E.coli and other coliforms. However, some commercial products will not detect the presence of the other bacteria associated with the deterioration of drinking water quality.

2.2.1 Hach P-A Test

Hach Presence-Absence broth contains the same ingredients as the P-A broth used by the MOEE but Hach tests are incubated up to 48 hours. The presence of coliforms, faecal coliforms, E.coli, faecal streptococci, Staphylococcus aureus and some Aeromonas species will cause Hach P-A broth to turn yellow. Coliforms are very likely present if tapping or gentle shaking of a positive (yellow) P-A bottle causes tiny gas bubbles to effervesce from the broth. Hach P-A broth with MUG (methylumbelliferyl-β-D-glucuronide) allows rapid detection of E.coli which most specifically indicate the presence of faecal contamination or sewage. Most strains of E.coli have the ability to hydrolyse MUG which causes the broth to fluoresce when exposed to long wavelength (365 nanometres) ultra violet (UV) light. This eliminates the reporting delay associated with confirmation tests.

2.2.2 Colilert, Colisure and Colibag

Colilert, Colisure and ColiBag (chromogenic response tests) detect coliforms based on detection of the enzyme β-D-galactosidase and E.coli based on detection of the enzyme β-D-glucuronidase. Test samples are incubated 24 - 28 hours. The production of β-D-galactosidase by coliform bacteria causes the culture media to change colour. In addition, most strains of E.coli produce β-D-glucuronidase which will hydrolyse MUG and cause the culture media to fluoresce when exposed to UV light.

ColiBag may detect Aeromonas species because they also produce the enzyme β-D-galactosidase but Colilert and Colisure are designed to inhibit growth of Aeromonas species. None of these products are designed to detect faecal streptococci, Staphylococcus aureus, Pseudomonas aeruginosa or Clostridium spp.

Quantitative results may be obtained with Colilert if it is used with a Quanti-tray. Although unnecessary for the initial screening of drinking water samples,

the Quanti-tray technique may have value if levels of E.coli and other coliforms need to be determined during sanitary surveys particularly since liquid, chromogenic response tests detect these bacteria with greater sensitivity than MF tests.

2.3 Performance

The MOEE uses a 3 day incubation period for its P-A test because this allows recovery of coliforms which may have been damaged by exposure to the disinfectants used to treat drinking water. Studies have shown that a number of P-A tests detect faecal indicator bacteria with similar ability. However, the bacteria were either not exposed to a disinfectant (4) or, when the bacteria were chlorine stressed, the comparisons did not include the P-A test used at the MOEE (5, 6).

In a recent MOEE evaluation, 110 samples of sewage or river water known to contain E.coli and other coliforms were added to flasks containing typical, chlorinated Toronto tap water. Parallel tests which included MF and various P-A methods were run at various times between 0-60 minutes exposure. The results of these tests revealed the following;

1) When used according to manufacturer's instructions and depending upon the P-A test, P-A methods detected E.coli and other coliforms as well as or better than MF tests.

2) Chromogenic response P-A (CRPA) tests often detected coliforms and E.coli more rapidly but in significantly fewer samples than the MOEE P-A test which uses a longer incubation period.

3) The number of samples found positive for coliforms and E.coli by CRPA methods approached the number of samples found positive for these bacteria by the MOEE P-A method when incubation of CRPA tests was extended to 48 hours (MOEE unpublished data).

When used according to manufacturer's instructions, all P-A products tested were at least as sensitive as currently used MF methods. When incubated beyond 24 hours, all P-A products were more sensitive than MF methods for detecting stressed E.coli and other coliforms.

The MOEE accepts that the commercial P-A test kit products referred to in this document may be used according to the specifications of the manufacturer. However, it is the opinion of the MOEE, at this time, that the performance of all of these P-A products and the role they fulfill in ensuring safe, high quality drinking water, may be

enhanced by extending the incubation period to at least 48 hours since it is most likely that any faecal indicator bacteria potentially present in samples collected from finished drinking water will have been stressed by exposure to a disinfectant.

References:

1) Clark, J.A. 1968. A presence-absence (P-A) test providing sensitive and inexpensive detection of coliforms, faecal coliforms and faecal streptococci in municipal drinking water supplies. Can. J. Microbiol. 14:13-18.

2) Jacobs, N.J. et al. 1986. Comparison of membrane filter, multiple-fermentation-tube, and presence-absence techniques for detecting total coliforms in small community water systems. Appl. and Environ. Microbiol. 51:1007-1012.

3) Ministry of Environment and Energy. 1994. Ontario Drinking Water Objectives.

4) Clark, J.A. and El-Shaarawi, A.H. 1993. Evaluation of commercial presence-absence test kits for detection of total coliforms, Escherichia coli, and other indicator bacteria. Appl. and Environ. Microbiol. 59:380-388.

5) McCarty, S.C. et al. 1992. Evaluating a commercially available defined-substrate test for recovery of E.coli. Jour. AWWA. 84(5):91

6) Covert, T.C. et al. 1992. Comparing defined-substrate coliform tests for detection of Escherichia coli in water. Jour. AWWA. 1992 84(50:98

3.0 RECOMMENDATIONS

The MOH and the MOEE agree that any of the above mentioned P-A methods may be used by trained staff at water treatment plants, municipal or private laboratories to screen drinking water for E.coli and other coliforms as part of the minimum requirement for testing samples routinely collected from community water supplies. P-A tests are at least as sensitive and many are more sensitive than currently used MF tests. However, based on the information provided, water works owners should decide;

1) whether or not they wish to use commercially available P-A tests at their facilities and,

2) which commercial P-A test they will use to detect undesirable bacteria in a manner that will allow the most effective management of their drinking water systems.

In addition, water works owners must collect samples to be analyzed for heterotrophic plate count (HPC) from a minimum of 25% of the sites sampled when a P-A test is used to analyze the water (ODWO 1994, Section 4.1.1). These samples should be sent to an accredited laboratory certified to perform bacterial tests on drinking water.

4.0 ACTION REQUIRED FOR POSITIVE P-A TESTS

The MOEE and the MOH agree that, when any P-A test produces a positive reaction, the laboratory (with permission of the treatment plant or distribution system owner) must notify the MOEE district abatement officer if test results indicate "deteriorating" (ODWO 1994, section 4.1.4) or "unsafe" water quality (ODWO 1994, section 4.1.2 and 4.1.3). In fact, the ODWO not withstanding, the MOEE and the MOH recommend that laboratories notify both the MOEE district abatement officer and the local Medical Officer of Health if test results indicate "unsafe" water quality in the form of either the persistence of coliforms or the presence of E.coli. Furthermore, water works staff must;

1) return immediately to the affected site(s), check disinfectant residuals in the area of the affected site(s) and, if necessary, raise the disinfectant level to at least the suggested minimum level (ODWO 1994, section 4.1.3),

2) collect additional "special" samples (ODWO 1994, section 4.1.3.1) from the area of the affected site(s) and send them for thorough analysis to an accredited laboratory certified to perform bacteriological tests on drinking water.

5.0 PROTOCOLS FOR SAMPLE COLLECTION

5.1 General Considerations

Aseptic techniques must be followed when handling sterile bottles and collecting the samples for microbiological analyses. Failure to do so may contaminate samples and compromise analytical results. The techniques described below should be followed closely in order to obtain reliable results.

Touch only the outer surface of the cap when opening the bottle. The inner lip of the bottle and cap liner must not come in contact with anything except the atmosphere and the water being sampled. If the inner surface of the cap or the mouth of the bottle are accidentally touched, the sample container must be considered contaminated and should not be used. Hold the cap in your fingertips while the sample is being taken. The cap must ***not*** be set down anywhere while the sample is being taken as this may result in contamination.

5.2 Sample Containers

Use only sterile glass or plastic bottles containing **sodium thiosulphate** to collect samples for bacteriological testing. The plastic seal on each container must be intact before sampling. Containers with loose or cracked seals should not be used.

Sodium thiosulphate is used to dechlorinate the sample and halt the disinfecting properties of chlorine. **A sample bottle containing sodium thiosulphate must be used if the water is known or suspected to contain a chlorine residual.**

5.3 Transport

Samples should be kept cool to minimize biological activity during transport to the laboratory by placing them into a container with ice or ice packs. **However, do not allow the sample water to freeze.** Samples should also be protected from direct light during transportation to the laboratory.

5.4 Holding times

The analysis of all chilled samples for bacteriological analysis should begin as soon as possible either on the day of sample collection or on the day after sample collection (maximum holding time 36 hours).

If samples for bacteriological analysis are not shipped in a chilled condition on ice, analysis should begin within 4 hours of sample collection.

5.5 Sample Volume

A 250 mL sample of water will provide adequate sample volume for all routine bacteriological analyses. However, additional samples may be required if the bacterial levels are expected to be very low, if extra tests are requested or if various tests must be performed at different laboratories.

5.6 Sampling Methodology

It is recommended that samplers adhere to the following procedures when collecting samples for bacteriological analysis.

5.6.1 Raw Surface Water Samples

Use a marker, which has water proof ink, to label the bottle with a sample number and/or description of the sampling site. Place the bottle into the clamp of a sampling pole and tighten the bottle in place. Avoid using a dipper, a sampler with a side-holder for other bottles, or any other sampling device which may contaminate the sample with bacteria from a source other than the water being collected. Remove the sample bottle cap and hold it in one hand. Do not place the cap down on any surface. With the other hand, use the sampling pole to reach out upstream over the water. Quickly lower the sample bottle into the lake, river or stream to about one meter below the surface with the mouth of the bottle facing into the current. Allow the water to fill the bottle but do not contaminate the sample with sediment. Remove the bottle from the water when bubbles are no longer observed coming from the bottle. After removing the bottle from the water, adjust the water level in the bottle to leave about 25 mL of head space. Carefully replace the cap on the bottle before removing it from a sampling pole clamp.

The sodium thiosulphate powder or pellet may be lost when collecting samples of surface water. Therefore, when collecting samples in an area where chlorine is likely to be present (e.g. treated sewage effluent or surface water near treated sewage effluent) aseptically transfer the water collected in the first bottle to a second sterile bottle containing sodium thiosulphate. This will ensure that there is sufficient sodium thiosulphate in the bottle to dechlorinate the sample.

5.6.2 Finished or Tap Water Samples

Sterile sample bottles containing sodium thiosulphate must be used to collect finished drinking water for bacteriological analyses.

Use a marker with water proof ink to label the bottle with a sample number and/or description of the site. Remove aerators, screens, hoses, etc. before taking drinking water samples from taps. Allow the water to run at full flow for at least two minutes before collecting the sample. The strong flow will clean out residual contamination around the orifice of the tap and provide a sample which is likely to be representative of the distribution system. After allowing the water to run for about 2 minutes, reduce the water flow. This will eliminate splashback which could result in contamination of the sample or loss of sodium thiosulphate while collecting the sample.

Remove the cap from the sample bottle with one hand. Handle the cap aseptically as described previously. Do not place the cap down on any surface. Hold the sample bottle with the other hand and place the open mouth of the bottle into the midstream of the water flow. Do not allow the mouth of the bottle to contact the tap or any other contaminated surface. Fill the bottle but leave about 25 mL of head space. Carefully replace the cap on the bottle.

When using containers supplied by distributors of commercial Presence-Absence (P-A) tests to collect drinking water samples for only these tests, fill the containers only to any 100 mL fill line marked on the container.

6.0 PROTOCOLS FOR THE USE OF COMMERCIAL PRESENCE-ABSENCE TESTS

N.B. Follow manufacturers instructions for storage of P-A media before use.

6.1 Hack P-A Broth

6.1.1 Analysis (according to manufacturer)

6.1.1.1 Label the Hach test bottle with the sample number and/or description of the site being tested.

6.1.1.2 Aseptically transfer water from the sample bottle into the Hach test bottle and fill to the 100 mL fill line.

6.1.1.3 Open the ampoule containing Hach P-A broth and aseptically transfer the contents into the bottle containing 100 mL of water sample.

6.1.1.4 Incubate the Hach P-A test at 35°C ± 0.5°C for up to 48 hours.

6.1.1.5 Every 24 hours, check the broth for turbidity (i.e. bacterial growth) and a change in colour from purple to yellow. If the broth remains purple on any day before the end of the incubation period, return the bottle to the incubator.

6.1.1.6 If the Hach P-A broth remains purple at 48 hours incubation, record the result as negative or "coliforms and E.coli not detected".

6.1.1.7 If the Hach P-A broth turns yellow and turbid with bacterial growth, check for the production of gas by tapping or gently shaking the bottle. If tiny gas bubbles effervesce profusely from the positive P-A broth, coliforms are very likely present and E.coli may be present.

6.1.2 Reporting

Whenever water from a single site or multiple sites causes Hach P-A broth to turn yellow and turbid with growth, record the result(s) for the site(s) as "adverse". The water quality is considered, at minimum, to be "deteriorating"

but could be "unsafe". Notify the MOEE abatement officer and the local Medical Officer of Health of the result(s).

N.B. In these cases, resample and take corrective action immediately because E.coli or other coliforms may be present, particularly if gas is produced in the positive P-A sample. The water quality may be "unsafe".

6.1.3 Additional Action

Whenever Hach P-A broth turns yellow, send staff immediately to the affected site(s). Check the disinfectant residual and increase it, if necessary, to at least the minimum requirement (ODWO 1994, section 4.1.3). Collect additional "special" samples and send them to an accredited laboratory certified to perform bacteriological tests on drinking water. Any laboratory which analyses the additional "special" samples collected in these cases should, at minimum, test the samples specifically for total coliforms, E.coli and heterotrophic plate count (HPC).

6.2 Hach P-A Broth with MUG

6.2.1 Analysis (according to manufacturer)

6.2.1.1 Label the Hach test bottle with the sample number and/or description of the site being tested.

6.2.1.2 Aseptically transfer water from the sample bottle into the Hach test bottle and fill to the 100 mL fill line.

6.2.1.3 Open the ampoule containing Hach P-A broth with MUG and aseptically transfer the contents into the bottle containing 100 mL of water sample.

6.2.1.4 Incubate the Hach P-A broth with MUG test at 35°C ± 0.5°C for up to 48 hours.

6.2.1.5 Every 24 hours, check the broth for a change in colour from purple to yellow. If the broth remains purple on any day before the end of the incubation period, return the bottle to the incubator.

6.2.1.6 If the Hach P-A broth with MUG remains purple at 48 hours incubation, record the result as negative or "coliforms and E.coli not detected".

6.2.1.7 If the Hach P-A broth with MUG turns yellow and turbid with bacterial growth, check for the production of gas by tapping or gently shaking the bottle. If tiny gas bubbles effervesce profusely from the positive P-A broth, coliforms are very likely present and E.coli may be present.

6.2.1.8 If the Hach P-A broth with MUG turns yellow at any time during the incubation period, take the bottle to a dark room or turn out the lights and use a long wavelength (365 nanometres) ultra violet lamp to check the broth for a blue fluorescence which is indicative of the presence of E.coli.

6.2.3 Reporting

6.2.3.1 **Single site:** Whenever water from a single site causes Hach P-A broth with MUG to turn yellow without blue fluorescence when exposed to UV light, record the result for that site as "adverse without E.coli". The water quality is considered to be "deteriorating". Notify the MOEE abatement officer of the result.

6.2.3.2 **Multiple sites:** Whenever water from multiple sites causes Hach P-A broth with MUG to turn yellow without fluorescence when exposed to UV light, record the results for those sites as "adverse without E.coli". The water quality is considered, at minimum, to be "deteriorating" but could be "unsafe". Notify the MOEE abatement officer and the local Medical Officer of Health of the results.

N.B. In this case, resample and take corrective action immediately because coliforms may be present at multiple sites, particularly if gas is produced in the positive P-A samples. The water quality may be "unsafe".

6.2.3.3 **Presence of E.coli:** Whenever water from any site causes Hach P-A broth with MUG to turn yellow and fluoresce when exposed to UV light, record the result(s) for the site(s) as " E.coli present". The water quality is considered to be

"unsafe". Notify the MOEE abatement officer and the local Medical Officer of Health of the result(s).

6.2.4 Additional Action

Whenever Hach P-A broth with MUG turns yellow or turns yellow and produces fluorescence, send staff immediately to the affected site(s). Check the disinfectant residual and increase it, if necessary, to at least the minimum requirement (ODWO 1994, section 4.1.3). Collect additional "special" samples and send them to an accredited laboratory certified to perform bacteriological tests on drinking water. Any laboratory which analyses the additional "special" samples collected in these cases should, at minimum, test the samples specifically for total coliforms, E.coli and heterotrophic plate count (HPC).

6.3 Colilert

6.3.1 Analysis (according to manufacturer)

6.3.1.1 Label the plastic Colilert test bottle with the sample number and/or description of the site being tested.

6.3.1.2 Aseptically transfer water from the sample bottle into the plastic Colilert bottle and fill the bottle up to the 100 mL fill line.

6.3.1.3 Open the packet containing Colilert powder and aseptically transfer the Colilert powder into the plastic Colilert test bottle containing 100 mL of water sample.

6.3.1.4 Cap the bottle and shake the contents until the powder dissolves

6.3.1.5 Incubate the inoculated Colilert test bottle at 35°C ± 0.5°C for 24 hours.

6.3.1.6 At the end of 24 hours incubation, check the Colilert test for a change in colour from colourless to yellow.

6.3.1.7 If the Colilert remains colourless at 24 hours incubation record the result as negative or "coliforms and E.coli not detected".

6.3.1.8 If the Colilert turns yellow during the incubation period and the yellow colour is stronger than the yellow colour in the comparator supplied by the manufacturer, take the bottle to a dark room or turn out the lights and use a long wavelength (365 nanometres) ultra violet lamp to check the Colilert for a blue fluorescence which is indicative of the presence of E.coli.

6.3.1.9 If the Colilert is slightly yellow at 24 hours incubation, follow the manufacturers instructions for comparison of the sample colour to the colour in the comparator and additional incubation.

6.3.2 Reporting

6.3.2.1 **Single site:** Whenever water from a single site causes the Colilert to turn yellow without blue fluorescence when exposed to UV light, record the result for that site as "coliforms present". The water quality is considered to be "deteriorating". Notify the MOEE abatement officer of the result.

6.3.2.2 **Multiple sites:** Whenever water from multiple sites causes the Colilert to turn yellow without blue fluorescence when exposed to UV light, record the results for those sites as "coliforms present". The water quality is considered to be "unsafe". Notify the MOEE abatement officer and the local Medical Officer of Health of the results.

6.3.2.3 **Presence of E.coli:** Whenever water from any site causes the Colilert to turn yellow with fluorescence when exposed to UV light, record the result(s) for the site(s) as " E.coli present". The water quality is considered to be "unsafe". Notify the MOEE abatement officer and the local Medical Officer of Health of the result(s).

6.3.3 Additional Action

Whenever Colilert turns yellow or produces fluorescence, send staff immediately to the affected site(s). Check the disinfectant residual and increase it, if necessary, to at least the minimum requirement (ODWO 1994, section 4.1.3). Collect additional "special" samples and send them to an accredited laboratory certified to perform bacteriological tests on drinking water. Any laboratory which analyses the additional "special" samples collected in these cases should,

at minimum, test the samples specifically for total coliforms, E.coli and heterotrophic plate count (HPC).

6.4 COLISURE

6.4.1 Analysis (according to manufacturer)

6.4.1.1 Label the Colisure test bottle with the sample number and/or description of the site being tested.

6.4.1.2 Aseptically transfer 100 mL of water from the sample bottle into the Colisure test bottle.

6.4.1.3 Transfer the contents of one Colisure culture medium bottle (2.6 gm) into the 100 mL of water sample.

6.4.1.4 Cap the bottle containing the water sample plus Colisure culture medium and mix the powder into the water by shaking the bottle.

6.4.1.5 Incubate the Colisure test at 35°C ± 0.5°C for 24-28 hours.

6.4.1.6 Check the broth at 24 hours incubation for a change in colour from yellow to red or magenta.

6.4.1.7 If the Colisure broth remains yellow at 24 hours, incubate the bottle for an additional 4 hours.

6.4.1.8 If the Colisure broth remains yellow at 28 hours incubation, record the result as negative or "coliforms and E.coli not detected".

6.4.1.9 If the Colisure broth turns red or magenta at any time during the 28 hour incubation period, take the bottle to a dark room or turn out the lights and use a long wavelength (365 nanometres) ultra violet lamp to check the broth for a blue fluorescence which is indicative of the presence of E.coli.

6.4.2 Reporting

6.4.2.1 **Single site:** Whenever water from a single site causes Colisure broth to turn red or magenta without fluorescence when exposed to UV light, record the result for that site as "coliforms present". The water quality is considered to be "deteriorating". Notify the MOEE abatement officer of the result.

6.4.2.1 **Multiple sites:** Whenever water from multiple sites causes Colisure broth to turn red or magenta without fluorescence when exposed to UV light, record the results for those sites as "coliforms present". The water quality is considered to be "unsafe". Notify the MOEE abatement officer and the local Medical Officer of Health of the results.

6.4.2.3 **Presence of E.coli:** Whenever water from any site causes Colisure broth to turn red or magenta with fluorescence when exposed to UV light, record the result(s) for the site(s) as "E.coli present". The water quality is considered to be "unsafe". Notify the MOEE abatement officer and the local Medical Officer of Health of the result(s).

6.4.3 Additional Action

Whenever Colisure broth turns red or magenta or produces fluorescence, send staff immediately to the affected site(s). Check the disinfectant residual and increase it, if necessary, to at least the minimum requirement (ODWO 1994, section 4.1.3). Collect additional "special" samples and send them to an accredited laboratory certified to perform bacteriological tests on drinking water. Any laboratory which analyses the additional "special" samples collected in these cases should, at minimum, test the samples specifically for total coliforms, E.coli and heterotrophic plate count (HPC).

6.5 ColiBag

6.5.1 Analysis (according to manufacturer)

6.5.1.1 Label the plastic ColiBag with the sample number and/or description of the site being tested.

6.5.1.2 Aseptically remove the top, perforated portion of the ColiBag and aseptically transfer water from the sample bottle into the ColiBag up to the 100 mL fill line.

6.5.1.3 Roll the top portion of the bag three or four turns down toward the bottom of the bag and secure the bag by bending the wire tabs over the rolled portion of the bag.

6.5.1.4 Allow the white sodium thiosulphate pellet to dissolve and then remove the white plastic separator strip from the outside of the bag and allow the water sample to mix with the culture medium powder.

6.5.1.5 Incubate the inoculated ColiBag at 35°C ± 0.5°C for 24 hours.

6.5.1.6 At the end of 24 hours incubation, check the broth for a change in colour from pale yellow to blue-green.

6.5.1.7 If the ColiBag broth remains pale yellow at 24 hours incubation, record the result as negative or "coliforms and E.coli not detected".

6.5.1.8 If the ColiBag broth turns blue-green during the incubation period, take the ColiBag to a dark room or turn out the lights and use a long wavelength (365 nanometres) ultra violet lamp to check the broth for a blue fluorescence which is indicative of the presence of E.coli.

6.5.2 Reporting

6.5.2.1 **Single site:** Whenever water from a single site causes Colibag broth to turn blue-green without fluorescence when exposed to UV light, record the result for that site as "adverse without E.coli". The water quality is considered to be "deteriorating". Notify the MOEE abatement officer of the result.

6.5.2.2 **Multiple sites:** Whenever water from multiple sites causes ColiBag broth to turn blue-green without fluorescence when exposed to UV light, record the results for those sites as "adverse without E.coli". The water quality is considered, at minimum, to be "deteriorating" but could be "unsafe". Notify

the MOEE abatement officer and the local Medical Officer of Health of the results.

NB. In this case, resample and take corrective action immediately because coliforms may be present at multiples sites and the water quality may be "unsafe".

6.5.2.3 **Presence of E.coli:** Whenever water from any site causes Colibag broth to turn blue-green with blue fluorescence when exposed to UV light, record the result(s) for the site(s) as "E.coli present". The water quality is considered to be "unsafe". Notify the MOEE abatement officer and the local Medical Officer of Health of the result(s).

6.5.3 Additional Action

Whenever ColiBag broth turns blue-green or produces fluorescence, send staff immediately to the affected site(s). Check the disinfectant residual and increase it, if necessary, to at least the minimum requirement (ODWO 1994, section 4.1.3). Collect additional "special" samples and send them to a laboratory accredited to perform bacteriological tests on drinking water. Any laboratory which analyses the additional "special" samples collected in these cases should, at minimum, test the samples specifically for total coliforms, E.coli and heterotrophic plate count (HPC).

7.0 QUALITY CONTROL

7.1 Staff must be fully trained in the use of any commercial P-A test method before they test water samples routinely collected from a community supply.

7.2 Record the lot number and expiry date for any batch of commercial P-A tests used to test a sample or series of samples. Commercial P-A tests must be used before the expiry date listed for the lot number. Do not use any P-A test beyond its designated expiry date.

7.3 A positive (Escherichia coli) and a negative (Klebsiella pneumoniae) control culture should be run to test the effectiveness of samples from each lot number of commercial P-A tests. Culti-loop bacterial cultures (Chrisope Technologies or equivalent) or Quanti-Cult bacterial cultures (Chrisope Technologies or equivalent) may be used to inoculate the P-A tests.

Prepare the control samples by adding 100 mL of water which does not contain E.coli or other coliforms to the P-A containers. Tap water, which has been boiled for 5 minutes and cooled to room temperature, may be used for this purpose. Add the P-A culture medium if it is packaged separately. Dip Culti-loops directly into the P-A tests until the material in the centre of the loop has dissolved. Follow manufacturers instructions for inoculating the Quanti-cult bacterial cultures into the P-A tests. Incubate the tests at 35 ± 0.5°C for 24 hours. E.coli should produce the appropriate, positive colour change in the P-A medium and cause a blue fluorescence in any medium containing MUG when it is exposed to UV light. K.pneumonia should cause the appropriate, positive colour change without fluorescence.

Culti-loops and Quanti-cult (E.coli ATCC 25922 and K.pneumoniae ATCC 13883) may be obtained from Unipath, 217 Colonnade Rd., Nepean, Ontario (613) 226-1318. Quanti-cult may also be obtained from Idexx Laboratories Inc., Cat. No. WKIT 101, phone 1-800-321-0207 for technical assistance).

Disinfect used Culti-loops, Quanti-cult (or equivalent) by autoclaving or placing them into a pail of disinfectant (described later section 8.1).

7.4 The fluorescence, in any P-A medium, caused by any drinking water sample should, at minimum, be compared to a negative control sample. Only samples which cause fluorescence which is greater than any fluorescence in the negative control should be considered positive for E.coli. A P-A test using 100 mL of E.coli free water or, preferably, a P-A test prepared using a negative (Klebsiella pneumoniae) control culture (Culti-loop, Quanti-Cult or equivalent) may be used as a negative control sample. Tap water, which has been boiled

for 5 minutes and cooled to room temperature, may be used for producing negative control samples.

7.5 Use a calibrated thermometer, traceable to a National Institute of Standards and Technology (NIST) thermometer, to ensure proper incubation temperature. Keep a record of the incubator temperature and record the incubator temperature daily.

8.0 DECONTAMINATION

8.1 P-A containers with growth

In a regular bacteriological laboratory, P-A containers which show evidence of bacterial growth should be decontaminated by autoclaving.

In the absence of an autoclave, P-A containers and contents which show evidence of bacterial growth should be disinfected before being discarded. The following method may be used to discard liquids and decontaminate containers.

Half fill a plastic pail with bleach solution of the following concentration; 100 mL of bleach (10-12% sodium hypochlorite) to each litre of tap water. Carefully pour the liquid from any P-A container, which shows either turbidity or a colour change as evidence of bacterial growth, into a toilet. Flush the liquid away. Submerge each empty P-A container into the bleach solution in the pail. Allow the containers to stand in the bleach solution for at least one hour. After 1 hour, carefully remove the containers from the bleach solution and rinse them with tap water. Carefully discard the bleach solution into a sink and flush the liquid away with plenty of tap water. Discard empty plastic P-A containers into a plastics recycling container if one is available. Otherwise, discard the plastic containers into the garbage. **NB. wear rubber or latex gloves and eye protection when handling bleach or any water containing a strong bleach solution.**

8.2 P-A containers without growth

Discard the liquid from any P-A container, which fails to show bacterial growth, into a sink and flush the liquid down the drain with lots of tap water. Rinse the empty plastic P-A containers with tap water and discard them into a plastics recycling container if one is available. Otherwise, discard the plastic containers into the garbage.

8.3 Leaks or Spills

Use a disinfectant to clean up leaks or spills from any P-A container which contains bacterial growth. Apply a generous amount of disinfectant (e.g. 5% Dettol or equivalent, or the above mentioned bleach solution) to the spill and the area in contact with the spilled material. Allow the disinfectant to contact the spill for at least 15 minutes, then mop up the spill with cloths or paper towels. Carefully wipe the contaminated outer surface of the P-A container with a fresh cloth or paper towel dampened with disinfectant. Wipe the container dry with a clean dry cloth or paper towel. Discard used cloths or paper towels into the garbage. **NB. wear rubber or latex gloves when mopping up spills and eye protection when handling any strong bleach solution. Wash hands thoroughly after cleaning up leaks or spills and after handling any P-A container which has bacterial growth.**

9.0 SAMPLES FOR HETEROTROPHIC PLATE COUNTS (HPC)

According to the ODWO, a minimum of 25% of all treated drinking water samples must be analyzed for heterotrophic plate counts (HPC) or background colonies on a total coliform membrane filter medium. All samples where no chlorine residual is detected must be analyzed for HPC (ODWO 1994, section 4.4.1). P-A tests do not produce background counts. Therefore, heterotrophic plate counts (HPC) are necessary when P-A tests are used to analyze the water for faecal indicator bacteria.

The MOEE recommends that treated drinking water from the plant effluent and each sampling site in the distribution system should be tested for HPC at least once per month. A sequential sampling technique may be used to test a sample collected from every fourth site sampled each week. If fewer than 4 samples are collected per week, at least one sample per week should be tested for HPC.

EXAMPLE

WEEK 1	Select samples 1, 5, 9, 13, etc. for HPC tests
WEEK 2	Select samples 2, 6, 10, 14, etc. for HPC tests
WEEK 3	Select samples 3, 7, 11, 15, etc. for HPC tests
WEEK 4	Select samples 4, 8, 12, 16, etc. for HPC tests
WEEK 5	Repeat week 1

Using this sequential method of sample testing, sampling sites representative of the entire system can be tested for heterotrophic plate count levels on a weekly basis while all sites are tested monthly. Alternatively, samples from all sampling sites may be submitted for testing once per month.

Currently acceptable heterotrophic plate count tests are easily contaminated by inexperienced staff. Therefore, the MOEE recommends that samples requiring heterotrophic plate counts should be sent to an accredited laboratory certified to perform bacteriological tests on drinking water.

10.0 RAW (SOURCE) WATER SAMPLES

P-A tests are not appropriate for testing raw (source) water. When tests on samples of raw (source) water are required, the samples are tested for levels of total coliforms and E.coli using quantitative techniques. Currently acceptable methods require costly equipment and the analytical techniques are not easily applied at small treatment plants. Raw (source) water samples should be submitted to an accredited laboratory certified to perform the required bacteriological tests unless the treatment plant has a facility which provides complete bacteriological testing of water.